Time effects on accuracy of homemade urease test in diagnosis of *H. Pylori*

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Abstract

To evaluate the effect of test duration on the sensitivity and specificity of positive urease test for the detection of Helicobacter pylori in patient with duodenal ulcer and gastropathy, and to evaluate the difference of the results of rapid urease test in patients with duodenal ulcer compared to patients with gastropathy. 117 patient"75 patient with duodenal ulcer group (A) and 42 patient with gastropathy group (B)" were included in the study, two sets of gastric biopsies "each contain one antral and one corpus biopsy" were obtained from each patient, one set used to obtain urease test which was read at 30 minutes, 2, 10 and 24 hour after biopsy insertion into the reagent, and the other set used for histopathological examination. 68/75 (90%) patients were tested positive for Helicobacter Pylori by histopathological examination in group (A), in comparison to 35/42 (83%) patient in group (B). The sensitivities of the urease test at 30 min, 2, 10 and 24 hours in group (A) were 45%, 62%, 73% and 99% respectively, corresponding figures for the specificity were 92%, 83%, 80% and 80% respectively. While in group (B) the sensitivities were 42%, 59%, 69%, and 95% respectively, and the specificity was 96%, 88%, 83%, and 81% respectively. The optimum duration of incubation of urease test for a good sensitivity was 24 hours; rapid urease test does not correlate to disease severity

Keywords: *Helicobacter Pylori*, Urease test, Duodenal ulcer, Gastropathy

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Introduction

Helicobacter pylori (*H. pylori*) is the micro-organism responsible for the most frequent bacterial infection worldwide. *H. pylori* infection affects about 50% of the world's population. In developing countries, the prevalence of infection is 90%, whereas in developed countries, the prevalence is below 40% [1]. The organism can be biochemically characterized as catalase, and urease enzyme positive. Urease appears to be crucial for its survival and colonization. Bacterial urease activity is clinically important because it forms the basis for several invasive and non-invasive tests to diagnose infection [2].

H. Pylori cause different diseases include chronic gast-ropathy, gastric and peptic ulcer, mucosal associated lymphomas and gastric carcinomas [3]. Endoscopic and non-endoscopic techniques are used to diagnose *H. pylori* infection. Endoscopic methods such as histology, rapid urease test, micro-biological culture and polymerase chain reaction (PCR), require endoscopy and are also known as biopsy-based tests. Non-endoscopic tests include stool antigen test, serology and urea breath test.

Some factors which influence the choice of a given testing strategy include sensit-ivity, specificity, the clinical circumstances and the cost-effectiveness of the test [4]. Notably, all these techniques have their own limitations [5, 6].

Rapid Urease Test (UT)

The RUT depends on the ability of *H. pylori* to produce urea as the basis for diagnosing infection. Biopsies that obtained during endoscopy are placed in agar or solution containing urea and a pH indicator. If urease is present, the urea is transform into carbon dioxide and ammonia, which increases the pH of the medium and causes a subsequent color change in the pH indicator.

The RUT produces a result in a range of minutes up to 24 hours. The RUT is cost effective, rapid, widely available, and highly specific [7].

False-negative results can occur with RUT due to decreased urease activity, which could be caused by a recent intake of antibiotics, proton pump inhibitors, bismuth compounds, or achlorhydria [8].

Different tests have been developed to evaluate the presence of urease activity and have been based on possible component of enzymatic reaction including those that assess pH change induced by the ammonia either as change in the color or using a pH meter. The initial test used phenol red in which the color indicator which changes from yellow to pink as gradual increase in the pH occur.

Other tests have used different indicators each with important advantage such ability to initiate the reaction at a lower pH such as 5.2 or thus decrease the activity of contam-inating mouth bacteria, many of which also contain abundant urease (e.g., HPfast®). Home-made is the simplest one and contain quantity of substrate in a test tube with a pH indicator [9, 10].

As for any enzymatic reaction, one must consider the factors that affect the reaction such as time and the concentration of substrate. A positive urease test requires approximately 105 *H. pylori* in the biopsy sample to change the color using an agar-based test such as the CLO test [10, 11].

This is generally not a problem as the concentration of *H. pylori* typically exceeds that minimum. The sensitivity of various UT tests as primary diagnostic tests is high and has been reported to vary between approx.-imately 80% and 100% and specificity between 97% and 99% [12-13].

Patient and methods

117 patients (69 male and 48 female) selected from those attending the endoscopy unit in Al-Hussein teaching hospital in Samawah for various dyspeptic symptoms during the period February 2011 - December 2013 were enrolled in this study.

Eligible criteria

Patients who have positive endoscopic findings (duodenal ulcer/gastropathy):

Absence of upper gastrointestinal malig-nancy, no prior gastric surgery, no acute upper gastrointestinal bleeding, and no consumption of non-steroidal anti-inflam-

matory drugs, proton pump inhibitors, anti-H. pylori antibiotics or bismuth preparations within four weeks of endoscopy. Patients were divided into two groups according to endoscopic findings:

Group A: 75 patients with duodenal ulcer (40 male and 35 female)

Group B: 42 patients with gastropathy (29 male and 13 female)

Upper gastrointestinal endoscopy with four gastric biopsies two from the antrum and two from the body for histopathological examination and urease test, was performed with fiber optic endoscope (EG-2490K model Pentax) after an overnight fasting.

Histopathology

One biopsy specimen from antrum and one from body were fixed in tubes containing 10% formaldehyde solution, each tube is labeled for patient's name. The specimen then stained by haematoxylin and eosin, and modified Giemsa to be used for histopathological examination.

The pathologist is considered the presence of spiral bacteria in the superficial mucous layer or along the luminal surface of the gastric epithelial cells as a positive test.

Urease test

One biopsy specimen from antrum, and one from body were used immediately for urease test detection, the biopsies specimen was put in tube, each contains 1 ml. of modified urea broth medium (the solution composed of Urea: 20 gm/ml, phenol red: 0.04 gm/l, KH2 Po4: 2gm/l Nacl: 5gm/ml) and labeled for time of taking biopsy. Each tube was maintained at ambient temperature and followed for change in color in four periods: at 30 minutes, 2 hrs, 10 hrs, and 24 hrs. The test was considered positive for H. Pylori when the color of urea broth changed from yellow-orange to pink, *H. pylori* infection was considered positive when histopathological result is positive.

Statistical analysis

Statistical analysis was performed with the SPSS, Version 20 (statistical Package for Social Sciences), 2011. Data analysis was done using T-test, P-value <0.05 was considered as a level of significance

Results

117 patients, 69 male (59%) and 48 female (41%) were enrolled in the study and subjected to various investigations for diagnosis of H. Pylori (histopathology, and rapid urease test). The age of the patient ranged from 18-68 years (mean age 39 years).

Age group distribution			
Age group in years	Patient		Total
	Duodenal Ulcer	Gastropathy	
< 20	9	1	10
20-29	18	13	31*
30-39	15	9	24*
40-49	12	11	23*
50-59	12	6	18
60-69	9	2	11
Total	75	42	117

* P<0.05

Table 1.
Age group distribution of patient with duodenal ulcer and gastropathy

Test	Result	Duodenal Ulcer	Gastropathy	Total
Histopathological examination	Positive	68	35	103
	Negative	7	7	14
	Total	75	42	117
UT at 30 minutes	Positive	35	12	47
	Negative	40	30	70
	Total	75	42	117
UT at 2 hours	Positive	42	18	60
	Negative	33	24	57
	Total	75	42	117
UT at 10 hours	Positive	63	31	94
	Negative	12	11	23
	Total	75	42	117
UT at 24 hours *	Positive	73	36	109
	Negative	2	6	8
	Total	75	42	117

* P=0.003

Table 2.
Result of H. Pylori diagnosis by histopathological examination and by UT (30 min, 2 hrs, 10 hrs, 24 hrs) in patient with duodenal ulcer and gastropathy.

Time interval	Sensitivity	Specificity	PPV*	NPV**
Duodenal Ulcer				
UT at 30 minutes	45%	92%	89	47
UT at 2 hours	62%	83%	89%	57%
UT at 10 hours	73%	80%	90%	66%
UT at 24 hours	99%	80%	90%	100%
Gastropathy				
UT at 30 minutes	42%	96%	79%	40%
UT at 2 hours	59%	88%	88%	55%
UT at 10 hours	69%	83%	89%	69%
UT at 24 hours	95%	81%	91%	95%
*PPV: Positive predictive value **NPV: Negative predictive value				

Table 3.
Sensitivity, specificity, PPV and NPP of UT at (30 minutes, 4 hours, 10 hours and 24 hours in patients with duodenal ulcer and gastropathy.

To evaluate the effect of time on the sensitivity and specificity of positive UT for detection of H. Pylori, four intervals (30 minute, 2 hrs, 10 hrs and 24 hrs) were studied. The detailed results of histopathological examination and time interval UT in duodenal ulcer and gastropathy are showed in table 2. The sensitivity, specificity, Positive and negative predictive value of UT are showed in table 3. In patient with duodenal ulcer the duration of incubation of UT increased the sensitivity from 30 minutes to 24 hrs, the sensitivity of UT at 30 minutes, 2 hrs, 10 hrs, and 24 hrs were 45%, 62%, 73%, 99% respectively, while the specificity was 92%, 83%, 80% and 80% for the corresponding period.

In patient with gastropathy, UT tend to increase sensitivity if the incubation period increased, the sensitivity of UT at 30 minutes, 2 hrs, 10 hrs, and 24 hrs were 42%, 53%, 61%, and 85%, respectively; while UT specificity tend to decrease with time from 69% at 30 min to 49% at 24 hrs.

Discussion

Dyspeptic patients infected with *H. pylori* are generally investigated by the physician during endoscopy. Detection of *H. pylori* infection on gastric biopsy specimens commonly uses rapid urease test because the results can be interpreted easily, rapidly and can give a result before patient is discharged from the endoscope room [14].

This study showed that in patients with duodenal ulcer the UT tended to increase in sensitivity if the incubation period was increased beyond 30 min to reach 99% at 24 hrs, and this was accompanied by little decrement in UT specificity in all four intervals; while in patients with gastropathy the UT tend to increase in sensitivity if incubation period was increased beyond 30 min, to reach 84% at 24 hrs, but this was accompanied by dropping in UT specificity beyond 2 hrs incubation from 69% to 49%. These results were similar to studies of Ho KY et al, [15] Lim LL, et al [16], and Goel et al, [17] who observed that the RUT tended to increase in sensitivity if incubation period

was increased but dropped in specificity with increasing the time of incubation. Ho KY et al also concluded that better results could be obtained if the RUT continued to be read over a 24 hrs period.

The sensitivities of RUT in patients with duodenal ulcer were similar to that of gastropathy in all four reading, and this results are similar to the study of Lewis JD [18]. who found that the results of RUT in gastritis is accurate for diagnosis but poorly correlated with disease severity.

The increase rate of false positive reading of RUT in patients with duodenal ulcer and gastropathy could be attributed to the instability of the reagent, or from autolysis of the tissue by chemical reaction or due to the presence of a contaminate with lower urease activity than *H. pylori* which need prolong incubation to produce a positive test such as *Gastrospirillum hominis, Pseudomonas and Proteus* [19-21].

Competing interests

The author declares that there is no conflict of interest.

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